PLANS OF SYNOPSIS

"TO THE STUDY OF DIVERSITY OF KERATINOPHILIC FUNGI AND SENSITIVITY EFFECT OF ETHNOMEDICINAL PLANTS ON THESE FUNGI PRESENT IN AJMER DISTRICT"

Submitted to

MAHARSHI DAYANAND SARASWATI UNIVERSITY

For

Ph.D (BOTANY)

By

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UNDER THE SUPERVISION & GUIDANCE OF

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APPENDIX – IV

MAHARSHI DAYANAND SARASWATI UNIVERSITY, AJMER

Format for Outline of Proposed Research work

(to be supplied in Six copies)

1. Name of Scholar (In English) :- RENU JANGID

   (In Hindi) :- रेनू जाँगिड

2. Title of Research Work :-

   (In English) **"TO THE STUDY OF DIVERSITY OF KERATINOPHILIC FUNGI AND SENSITIVITY EFFECT OF ETHNOMEDICINAL PLANTS ON THESE FUNGI PRESENT IN AJMER DISTRICT"**

   (In Hindi) : अजमेर जिले में उपस्थित केरॉटिन युक्त कवकों की विविधता व इन कवक पर औषधिय पादपों के सचेतन प्रभाव का अध्ययन।

3. Location

   a. Institution/Department where the work is to be done : DEPARTMENT OF BOTANY SPC GOVERMENT COLLEGE, AJMER RAJASTHAN 305001

   b. Geographical area of Investigation, if any : AJMER RAJASTHAN

Annexure – I

Introduction

Keratinophilic fungi are ecologically an important group of fungi which are found in soil. During the past years, many researchers reported and isolated Keratinophilic fungi around the world. Keratinophilic fungi are involved in the breakdown of keratinaceous substrates and are present in the environment worldwide. Keratinophilic fungi have ability to degrade keratin. Keratin is the cornified part of the epidermis of vertebrates, which includes feather, hair, nail, horn, wool etc. After death of animal or insects the remain are added to the soil. A lot of substrate rich in keratin is also added to the soil. Keratin can be divided into soft and hard keratin depending on the cystine content in it. The molds capable of attacking keratinized tissues are termed as keratinophilic fungi. These belong to hyphomycetes and several other taxonomic groups hyphomycetes include dermatophilic fungi and a variety of non- dermatophilic keratinophilic fungi. Most of species of dermatophytes are anthropophilic or zoophilic in their natural habitat, while some occur in soil as saprophytes and are termed geophilic, for example *Microporum gypseum* and *Trichophyton terrestre*. Non-dermatophytic keratinophilic fungi, including species of *Chrysosporium* and other genera of fungi, are known to occur as saprobes in soil. Some keratinophilic fungi are called dermatophytes because of their capacity to parasitize keratinic tissues in man and animals.

Dermatophytes are fungi which parasitize keratinized tissues in man and animal and causes dematophytosis. The dermatophytosis, which is also known as cutaneous mycosis are infections of keratinized epidermal tissues of human beings. All the dermatophytes are keratinophilic in nature. Dermatophytes are one of the most frequent skin diseases of human, pets and livestock. The disease is widely distributed all over the world with various degrees and more common in men than in women.

On the basis of the morphology and sexual reproduction dermatophytes are included in arthroderma or in hannizzia, two closely related genera within the family gymnoascaceae; order eurotiales in the ascomycotina recognized three

genera of dermatophytes: *Microsporum, Trichophyton* and *Epidermophyton,* which are based on clinical rather than mycological observations.

Skin, hair, nail, and subcutaneous tissues in human and animal are subjected to infection by several organisms, mainly fungi named dermatophytes and cause dermatophytoses. Dermatophytoses are one of the most frequent skin diseases of human, pets and livestock.

A wide variety of dermatophytes have been isolated from animals, but a few zoophilic species are responsible for the majority of the cases, viz. *Microsporum cavis, Trichophyton mentagrophytes, Trichophyton equinum* and *Trichophyton verrucosum,* as also the geophilic species *Microsporum gypseum*.

Different treatment has been recommended to control dermatophytes but recently the use of some natural plant products has been emerged to inhibit the causative organism. Plant extract has been used traditionally to treat a number of infectious diseases including those caused by bacteria, fungi, protozoa and viruses. Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Medicinal plants with antimicrobial activity are known to offer protection against various bacterial, viral and other diseases and also find industrial applications. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied drugs medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. Although hundreds of plant species have been tested for sources for antimicrobial drugs. Different biologically active substances have been isolated from plant extract, among them organosulphur compounds such as allicin, azones and diallyltrisulfide. Most important biologically active compound found in plant extract and show antifungal activity.

Objectives

Objectives of the proposed study are as given below :-

1. To study of Keratinophilic fungi in soil microflora.
2. In vitro study of anti dermatophytic activity of some plant extracts.
3. To study the comparision of antifungal or anti dermatophytic properties of different organic plant extract.
4. Development of more effective and less toxic antifungal agent is required for the treatment of dermatophytosis.
5. Plants and their extraction will be prepared for use in medicines against infectious diseases.

Annexure - II

Review of literature

- AP Garg et al (1985) studied the ecology of Keratinophilic fungi. In the soil Keratinophilic fungi are responsible for the breakdown of any keratin containing wastes such as hair, fur and feather. These fungi therefore play a significant role in the breakdown of soil debris. In this review we have evaluated the information available on ecological factors governing the distribution and growth of keratinolytic fungi.
- Qureshi S et al (1997) studied In vitro evaluation of inhibitory nature of extracts of 18-plant species of Chhindwara against 3-keratinophilic fungi. Effect of extract of 18 plant species, viz., *Acorus calamus, Adhatoda vasica, Amomum subulatum, Andrographis paniculata, Boerhaavia diffusa, Cassia occidentalis, Centella asiatica, Cymbopogon citratus, Hemidesmus indicus, Hyptis suaveolens, Malvestrum sp., Passiflora edulis, Pergularia daemia, Peristrophe bicalyculata, Shuteria hirsuta, Solanum nigrum,and Tecoma stans* on the growth of *Microsporum gypseum, Chrysosporium tropicum and Trichophyton terrestre* was evaluated and discussed. The sensitivity of the keratinophilic fungi was evaluated by dry-weight method. The maximum inhibition of mycelial growth was shown by *M. gypseum* (86.62%) followed by *T. terrestre* (81.86%) and *C. tropicum* (74.06%) when treated with S. hirsuta whereas the minimum inhibition was exhibited by *M. gypseum (0.29%), C. tropicum (0.16%) and T. terrestre (1.76%)* when tested with the extract of *P. edulis, A. vasica and B. diffusa* respectively.
- Deshmukh and Verekar (2006) studied the occurrence of dermatophytes and other keratinophilic fungi from the soils of Himachal Pradesh (India). A total of 122 soil samples were examined, of which 101 samples were positive for keratinophilic fungi and related dermatophytes.
- Fardos M. Bokhari (2009) studied Antifungal activity of some medicinal plants used in Jeddah, Saudi Arabia. In this research develop more effective and less toxic antifungal agents for the treatment of dermatophytosis.

Plant and their extraction preparations have been used as medicines against infectious diseases.

- Esmat malek et al (2013) studied The Isolation of Keratinophilic Fungi and aerobic actinomycetes from park soils in Gorgan, North of Iran. In this study, they surveyed the city park soils of Gorgan (a northern province of Iran) to determine the identities and diversity of soil aerobic actinomycetes, keratinophilic and non-keratinophilic fungi.
  This study showed that the collected soil from studied areas was rich of keratinophilic fungi and Actinomycetes, therefore hygiene protocol should be taken to prevent the spread of pathogenic and saprophytes fungi in the environment of susceptible person.

- Deshmukh and Verekar (2014) studied Incidence of Keratinophilic fungi from selected soils of Vidarbha region of Maharashtra State, India One hundred and fifty samples were collected from eleven districts of Vidarbha region of Maharashtra state and screened for the presence of keratinophilic fungi using hair baiting technique for isolation. Seventy-one isolates were recovered and identified. The cultures were identified using macro- and micromorphological features. Their identification was also confirmed by the BLAST search of sequences of the ITS1-5.8S-ITS2 rDNA region against the NCBI/Gene bank data and compared with deposited sequences for identification purpose.
- Hafez et al studied the occurrence of keratinophilic fungi in sewage sludge from Egypt The keratinophilic fungi of 40 sewage sludge samples from Upper Egypt were studied using a goat hair-baiting technique. 43 species representing 22 genera were isolated, 17 species of which were dermatophytes and closely related fungi: *Chrysosporium* state of *Arthroderma tuberculatum, C. asperatum, C. georgii, C. indicum, C. keratinophilum, C. pseudomerdarium, C. queenslandicum, Chrysosporium* state of *Thielavia sepedonium, C. tropicum, Microsporum cookei, M. gypseum, Myceliophthora* anamorph of *Corynascus novoguineensis, M. vellerea* and *Trichophyton terrestre.* 26 species of cycloheximide resistant fungi were collected and these included members of *Acremonium, Aspergillus, Alternaria, Chaetomium, Cladosporium, Cunninghamella, Emericella, Fusarium, Geotrichum, Penicillium* and others.

Annexure – III

Research gaps identified in the proposed field of investigation

The occurrence of dermatophytes in soil was reported for the first time by Vanbreuseghem using the hair bait technique. Since then studies on the isolation of Keratinophilic fungi from soil samples(Egypt 2005, Himachal Pradesh 2006, Lonar 2006, Iran 2013, Maharashtra 2014, Jamaika 2014 etc.) have been investigated throughout the world.

The Present research are aim to investigate Diversity of Keratinophilic fungi and their sensitivity to some Ethnomedicinal plants occur in Ajmer district.

Annexure - IV

Major Hypothesis, If Any

Keratinophilic fungi are an ecologically important group of fungi that decompose keratin protein. Some of these fungi are well-known dermatophytes and cause superficial cutaneous infections (dermatophytoses) of keratinized tissue (skin, hair, and nails) of human and animals. Dermatophytoses are one of the most frequent skin disease. The use of some natural plant products has been emerged to inhibit causative organism.

In the present study we will discover some plant extract which will have antifungal activity occurring in different sites of Ajmer district (Rajasthan). These extract can be effective against Keratinophilic fungus. They are safe to human and the ecosystem than the chemical antifungal compounds, and can easily be used by public.

Annexure - V

Research Methodology

Collection of soil samples and baits: Thirty six soil samples will be collected randomly from 12 garbage dumping sites from different sites of Ajmer district. Before collection of soil samples, superficial debris and other vegetative materials will removed from the soil surface. Loosened soil (approximately 500g) will taken from the surface layer of each site to a depth of 2-5cm. Soils will collected in sterile plastic bags and sealed on the spot. Samples will brought to the laboratory and used immediately or stored overnight.

Isolation & identification of keratinophilic fungi:
Keratinophilic fungi will isolate by the hair baiting technique. The soil samples and moistened with sterile distilled water are baited by burying sterile Keratinous baiting the soil. These dishes will incubated at room temperature (Period of 4 weeks). After observing the mycelial growth on the baits, isolates will culture on Sabouraud's Dextrose Agar (SDA) medium supplement with streptomycin (30 mg/l).

Hair Baiting Technique

1. Moist chamber will prepared using sterile Petri dishes and blotting paper
2. Sterile Petri dishes will half fill with soil sample
3. 2-3cm short strands of sterilized defatted baits will spread over soil surface
4. 10-15 ml of sterile water will added to the soil to facilitate germination of fungal spore
5. Petri dishes will then incubated at room temperature.
6. Plates will examined periodically for the development of mycelium

Medicinal plant materials: Samples of five Medicinal plants, i.e. *Catheranthus roseaus, Sida cordifolia, Zizyphus sp., Lantana sp.* and *Nerium sp.* Will be collected from various regions of Ajmer district.

Preparation of aqueous and organic medicinal plant extracts: For aqueous and organic extraction, 10 grams of each sun-dried medicinal plant material, will cut into small pieces and then macerated by blender 1–2 mm separately and the powder produce will blended with 100 ml of either distilled water (cold or hot) or organic solvent (ethyl alcohol, methanol, n-butanol, ethyl acetate or chloroform), (1:10 w/v). Then, they will be extracted under cold conditions for 24 hrs. The resultant extract will filtered through a glass wool filter and then rinsed with a small quantity (about 30 ml) of 96% ethyl alcohol. The extracts solutions will evaporated under reduced pressure at 40 °C. Subsequently, the extracts will dilute by distilled water and stored in the deep freezer at -10 °C and later lyophilized in a freeze dryer.

Antimicrobial activity

Antimicrobial activity of the above mention extracts are determine, using the agar well diffusion assay method as described by Holder and Boyce (1994). Dimethyl sulfoxide (DMSO) will be used as a negative control and Griseofulvin will be used as a positive control. The plates will be kept in triplicates and will be incubated at 37°C. The antimicrobial activity are taken on the basis of diameter of zone of inhibition, which will measured after 7 days of incubation and the mean of three readings is present. The presence of inhibition of the treated fungus will calculate using Griseofulvin as standard (100% inhibition). The plant extract and the standard antifungal agents will be dissolved in DMSO, 100% biologically inert substances.

Annexure - VI

References

- Abdel-Fateh, H.M., Moubasher, A.H. Maghazy, S.M(1982). Keratinophilic fungiin Egyptian soils. Mycopathologia 79:49-53
- Abdulmnoniem M.A., saadab A.M.A, (2006). Antifungul activity of Saudi pants used in traditional medicine. Asian J. Plant Sci., 5:907-909.
- Aly A.A., Omar S.A., Zayed S.M.E., Mansour MTM, (2000). Use of saponin-containing A triplex nummularia to suppress damping of cotton seedling. J. Agric. Sci. Mansura Uni., 25:7621-7631.
- Ajello, L.(1960). Geographic distribution and prevalence of dermatophytes. Ann. N.Y.A cad. Med. Sci., 89:30-38.
- Anbu Pariasamy, Hilda A., Gopinath S(2004). Keratinophilic fungi of poultry farm and feather dumping soil in Tamilnadu, India. Mycopathologia;158(3):303–309.
- Ali-Shtayeh M.S., Jamous R.M.F.(2000). Keratinophilic fungi and related dermatophytes in polluted soil and water habitats. In: Kushwaha RKS, Guarro J, editors. Biology of dermatophytes and other keratinophilic fungi. Revista Iberoamericana de Micologia Spain. 2000. pp. 51–59.
- Deshmukh S.K., Agrawal S.C., Jain P.C.(2000) Isolation of dermatophytes and other keratinophilic fungi from soils of Mysore(India) Mycoses;43:55–57.
- Deshmukh S.K., and Verekar S.A.(2006). "The occurrence of dermatophytes and other keratinophilic fungi from the soils of Himachal Pradesh (India)," Czech Mycology, vol. 58, no. 1-2, pp. 117–124.
- Deshmukh S.K. (2002). "Incidence of dermatophytes and other keratinophilic fungi in the glacier bank soils of the Kashmir valley, India," Mycologist, vol. 16, no. 4, pp. 165–167.
- Deshmukh S.K.(2002). "Isolation of dermatophytes and other keratinophilic fungi from Karnala bird sanctuary, Maharashtra (India)," Journal of Basic and Applied Mycology, vol. 1, no. 2, pp. 194–196.

- Deshmukh S.K. (2004). "Isolation of dermatophytes and other keratinophilic fungi from the vicinity of salt pan soils of Mumbai, India," Mycopathologia, vol. 157, no. 3, pp. 265–267.
- Deshmukh S.K., and Agrawal S.C.(2003). "Isolation of dermatophytes and other keratinophilic fungi from soils of Jammu, India," Mycoses, vol. 46, no. 5-6, pp. 226–228.
- Fillipello Marchisio V. (1968). Keratinolytic and keratinophilic fungi of childrens sandpits in the city of Turin. Mycopathologia ;94:163–72.
- Ghosh G.R., and Bhatt S. (2000). "Keratinophilic Fungi from Chilka Lake-side Soil Orissa (India)," Indian Journal of Microbiology, vol. 40, no. 4, pp. 247–254.
- Gupta R, Garg A.P. (1991). Isolation of keratinophilic fungi from meerut with particular reference of soil pH. Acta Bot Indica ;1:283–285.
- Ghosh G.R., Biswass S.B. (1995). Keratinophilic fungi from Bharatpur bird Sanctuary. Indian J Microbiol ;35(2):153–159.
- Gugnani H.C., Sharma S., and Wright K. (2014). "A preliminary study on the occurrence of keratinophilic fungi in soils of Jamaica," Revista do Instituto de Medicina Tropical de Sao Paulo, vol. 56, no. 3, pp. 231–234.
- Garg K. (1966). "Isolation of dermatophytes and other keratinophilic fungi from soils in India.," Sabouraudia Journal of Medical and Veterinary Mycology, vol. 4, no. 4, pp. 259–264.
- Gugnani H.C., Sharma S., Gupta B., and Gaddam S. (2011) "Prevalence of Keratinophilic fungi in soils of St. Kitts and Nevis".
- Gugnani H.C., Sharma S., and Wright K. (2014) "A preliminary study on the occurrence of Keratinophilic fungi in soils of Jamaica."
- Jain N., Bhadauria S., Sharma M., Kumar P. (1999). Keratinophilic and related fungal flora of Jaipur II. J Phyto Res. 12(1–2):105–106.
- Jain N., Sharma M., Saxena V.N. (2008). Clinicomycological profile of dermatophytosis in jaipur, Rajasthan. Indian J Dermotal Venerol and Leprol. 2008;74:274–275.
- Jain N., Sharma M. (2011). Distribution of dermatophytes and other related fungi in Jaipur city with particular reference to soil pH. Mycoses. 54(1):52–58.
- Jain P.C., and Agrawal S.C. (1979). "Some additions to Indian Malbranchea," Kavaka, vol. 7, pp. 69–72.

- Jain N., and Sharma M. (2011). "Distribution of dermatophytes and other related fungi in Jaipur city, with particular reference to soil pH," Mycoses, vol. 54, no. 1, pp. 52–58.
- Kumar S., Deshmukh, and Verekar S.A. (2014). " Incidence of Keratinophilic fungi from Selected Soils of Vidarbha Region of Maharastra State, India.
- Kushwaha R.K.S. (2000). The genus Chrysosporium, its physiology and biotechnological potential. In: Kushwaha RKS, Guarro J, editors. Biology of dermatophytes and other keratinophilic fungi. Bilbao, Spain: Rev Iberoamericana Micol. pp. 66–76.
- Kushwaha R.K.S., and Agarwal S.C. (1976). "Some keratinophilic fungi and related dermatophytes from soils," Proceedings of the Indian National Science Academy, vol. 42, no. B, pp. 102–110.
- Kaben U. (1963). Die Isoliereing von kertinomyces ajelloi, Trichophyton terrestre, Microsporum gypseum and Microsporum cookie aus dem Erdboden. Bull Pharm Res Inst (Osaka) ;45:11–20.
- Katiyar S., and Kushwaha R.K.S. (2001). "Human hair colonizing fungi in water sediments of India," Mycopathologia, vol. 152, no. 2, pp. 81–84.
- Lysková P. (2007). "Saprotrophic microscopic fungi and dermatophytes accompanying infections of the skin and nails of patients in the Moravian-Silesian Region (Czech Republic)," Czech Mycology, vol. 59, pp. 125–137.
- Mahmoudabadi A.A., Zarrin M. (2008). Isolation of dermatophytes and related keratinophilic fungi from the two public parks in Ahvaz. Jundishapur J. Microbiol., 1: 20-23 20
- Maoz M., Neeman I. (1998). Antimicrobial effects of aqueous plant extracts on the fungi Microsporum canis and Trichophyton rubrum and on three bacterial species. Lett. Appl. Microbiol., 26: 61-63.
- Natarajan V., Venugopal P.V., Menon T. (2003). Effect of azadirachta indica (neem) on the growth pattern of dermatophytes. Indian J. Med. Microbiol., 21: 98-10.
- Ogbonna C.I.C., Pugh G.J.F. (1987). Keratinophilic fungi from Nigerian soil. Mycopathologia. 99:115–118.
- Oyeka, A. (2000). "Trichophyton mentagrophytes a keratinophilic fungus," in Biology of Dermatophytes and other Keratinophilic Fungi, R. K. S. Kushwaha and J. Guarro, Eds., pp. 60–65, Revista Iberoamericana de Micologia, Bilbao, Spain.

- Patwardhan N., Dave R. (1999). Dermatomycosis in and around Aurangabad. Indian J Path Microbiol. 42(4):455–462.
- Prasad C.S., Shukla R., Kumar S., Dubey N.K. (2009). In vitro and in vivo antifungal activity of essential oils of Cymbopogon martini and Chenopodium ambrosioides and their synergism against dermatophytes. Mycoses, 3: DOI: 10.1111/j.1439-0507.2008.01676.
- Padhye, A, Misra,S.P., and Thirumalachar, M.J. (1966). "Occurrence of soil inhabiting dermatophytes and other keratinophilic fungi from soils in Poona," Hindustan Antibiotics Bulletin, vol. 9, no. 2, pp. 90–93.
- Pakshir K., Ghiasi M., Zomorodian K., and Gharavi A.R. (2013). "Isolation and molecular identification of keratinophilic fungi from public parks soil in Shiraz, Iran," BioMed Research International, vol. 2013, Article ID 619576, 5 pages, 2013.
- Randhawa H.S., and Sandhu R.S. (1965). "A survey of soil inhabiting dermatophytes and related keratinophilic fungi of India," Sabouraudia Journal of Medical and Veterinary Mycology, vol. 4, no. 2, pp. 71–79.
- Saxena P., Kumar A., and Shrivastava J.N. (2004). "Diversity of keratinophilic mycoflora in the soil of Agra (India)," Folia Microbiologica, vol. 49, no. 4, pp. 430–434.
- Sur and Ghosh G.R. (1980). "Keratinophilic fungi from Orissa, India I: isolation from soils," Sabouraudia Journal of Medical and Veterinary Mycology, vol. 18, no. 4, pp. 269–274.
- Singh, Kushwaha R.K.S., and Parihar P. (2009). "Keratinophilic fungi in soil of potted plants of indoor environments in Kanpur, India, and their proteolytic ability," Mycoscience, vol. 50, no. 4, pp. 303–307.
- Kaul S., and Sumbali, (1997). "Keratinolysis by poultry farm soil fungi," Mycopathologia, vol. 139, no. 3, pp. 137–140.
- Singh B.S., Singh D.V., Singh B.G., Singh R.J. (1990). Incidence of keratinophilic fungi and related dermatophytes in Agra soil.
- Sharma M., Williamson D. (1984). Keratinophilc fungal flora of Jaipur district. Biol Bull India Bhagalpur. 6:195–204.

- Singh and Kushwaha R.K.S. (2010). "Dermatophytes and related keratinophilic fungi in soil of parks and agricultural fields of Uttar Pradesh, India," Indian Journal of Dermatology, vol. 55, no. 3, pp. 306–308.
- Singh I., Mishra A., and Kushwaha R.K.S. (2009). "Dermatophytes, related keratinophilic and opportunistic fungi in indoor dust of houses and hospitals," Indian Journal of Medical Microbiology, vol. 27, no. 3, pp. 242–246.
- Tsang P., Hopkins T., Jimenez-Lucho V. (1996). Deep dermatophytosis caused by Trichophyton rubrum in a patient with AIDS. Vol. 34, No. 6: 1090-1091.
- Tripathi N., Kushwaha R.K.S. (2005). Indian keratinophilic fungal flora. In: Deshmukh SK, Rai MK, editors. Fungi: Diversity and Biotechnology. Jodhpur: Scientific Publishers. pp. 31–62.
- Verma T.N., Sinha B.K., and Das U.L. (1982). "Isolation of keratinophilic fungi from soil in Bihar (India)," Mykosen, vol. 25, no. 8, pp. 449–452.
- Vanbreuseghem R. (1952). Technique biologique pour! isolements des dermatophyte du soil (Biological technique for the isolation of dermatophytes from the soil. Ann Soc Belge de Med Tech. 1952;32:173–178.
- Vidyasagar G.M., Hosmani N., and Shivkumar D. (). "Keratinophilic fungi isolated from hospital dust and soils of public places at Gulbarga, India.
- Verekar S.A., and Deshmukh S.K. (2006). "Incidence of keratinophilic fungi from caves around Mumbai, Maharastra (India)," Journal of Basic and Applied Mycology, vol. 5, pp. 62–64.
- Zaki S.M., Mikami Y., Karam A.A., El-Din Youssef Y.A. (2005). Keratinophilic fungi recovered from muddy soil in Cairo vicinities. Egypt. Mycopathologia. 2005;160:245–281.

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25.5.16

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Outline Approved.

Name and Signature of Supervisor with date & seal.